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## THE SANITARY SIGNIFICANCE OF BODY CELLS IN MILK.\*

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Cow's milk contains a certain number of cells derived from the tissues of the udder whose nature has been variously interpreted by different investigators. The presence of these tissue cells in colostrum milk, where they have been spoken of as colostrum corpuscles, has been recognized for a long time. The usual statement in textbooks and elsewhere is that these corpuscles are abundant for a few days after calving and then disappear. It has remained for the sanitarians of the last decade to show that tissue cells occur not only in colostrum milk but in all milk.

Because of the occurrence of enormous fluctuations in numbers of these cells, it has been thought that these fluctuations have a sanitary significance. Many health officers who believe that large numbers of these cells indicate pathological conditions have condemned and still condemn milk on this basis. Thus the problem of interpreting the real significance of their presence becomes an important one.

The first person to direct attention to the presence of the cells in ordinary milk was Dr. Stokes of Baltimore, who published the first article discussing their sanitary significance in 1897.<sup>1</sup> He looked at the problem presented by their presence from the standpoint of the medical man and health officer, and, because he recognized some of them as white blood corpuscles, he naturally thought of them as "pus" cells, as these cells occur in great abundance in inflamed regions and in pus. Unfortunately, too many of the later studies of the cells in milk have been made from the same standpoint, a standpoint which has its limitations as well as its advantages. The particular limitation has been the tendency of other health officers to accept Dr. Stokes's interpretation of these cells as "pus" cells without question, and to talk about the presence of "pus" in the milk. Dr. Stokes tells me that he, himself, now regards this interpretation of their nature as unfortunate. Moreover, health officers are usually concerned with the examination of market milk samples, and are rarely in a position to trace abnormal conditions in any satisfactory way to their source. In many examinations of market milk abnormally large numbers of these cells have been found.

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<sup>1</sup> *Med. News*, 1897, 71, p. 45.

Frequently the same sample has shown large numbers of long chain streptococci of a type generally regarded as pathogenic. It has been assumed that the two were associated as cause and effect, the streptococci coming from pathological udders which were discharging large numbers of "pus" cells because of the presence of the streptococci. This assumption is possibly correct but rests on very insufficient data. Investigators have frequently traced their samples back to the herds from which the milk was obtained and, finding there a cow with garget, or a three-teated cow, or a fresh cow whose milk had been saved to the end of her lactation period, or suckling calves, or a poor, scrawny cow, have assumed, without tests, that these animals were responsible for the increased cell counts which they found. Inasmuch as it is uncommon to find a herd which does not have such animals, it is not surprising that they should have been found in practically all of these cases.

Moreover, almost all of the determinations of the numbers of cells present have been made by methods which were modifications of the first one used by Dr. Stokes; that is, the cells were counted in the sediment obtained by centrifuging, either by making stained dried smears from the sediment, or by counting the cells in the sediment by means of the Thoma-Zeiss blood cell counter.

Inasmuch as Professor Prescott and myself<sup>1</sup> showed some three years ago that centrifuge slimes sometimes contained as many as one-half of the cells and sometimes not more than one-fortieth of them even when the milk was centrifuged under the same conditions, one can readily see why the cell counts obtained in this way are not now regarded as accurate. Other investigations have shown that practically all of the cells rise with the cream in the case of gravity-raised cream and that the number of cells precipitated by centrifuges or separators is influenced by preliminary heating of the milk,<sup>2</sup> the speed of revolution, and probably other factors which make it impossible to secure consistent results even with the same centrifuge or separator.<sup>3</sup>

The following method has been used in all of the work referred to here: a small, measured drop of milk was taken directly without centrifuging and smeared over a known area on a glass slide, dried, stained, and the cells counted with a microscope. In this way the chance of throwing away from 50 per cent to 97.5 per cent of the cells has been eliminated. This method has its limitations and it is impossible to get duplicate tests to check absolutely, but in the hands of a reasonably careful person, there is no difficulty in getting duplicate cell counts to agree much better than duplicate plate counts for bacteria. There is no question but that the results secured represent a close approximation to the truth.

#### NATURE AND ORIGIN OF THE CELLS.

The first problem to be solved in regard to these cells is their nature and source. Three views have been held. They have been regarded (1) as leukocytes or white blood corpuscles, and because these cells occur in large numbers in inflamed regions and in "pus," they have frequently been thought of as "pus" cells;

<sup>1</sup> *Jour. Infect. Dis.*, 1910, 7, p. 632.

<sup>2</sup> *24th Ann. Rept., Wis. Agric. Exper. Sta.*, 1907, p. 231.

<sup>3</sup> *Arch. f. Hyg.*, 1911, 75, p. 383.

(2) as epithelial cells and fragments of such cells derived from the epithelial lining of the secreting portion of the udder; (3) as a mixture of both kinds of cells.

All investigators admit that both white and red blood corpuscles occur under unusual conditions when blood makes its way directly into the milk.

In my opinion there is no question but that the third view is correct for the following reasons:

1. Because of the appearance of the cells. Certain of these cells are exactly like polymorphonuclear and polynuclear leukocytes. This type of cell has a nucleus of an unmistakable kind. No other cell in the body has one like it. These cells likewise stain characteristically in blood stains. Others of the cells closely resemble epithelial cells and may even occur in groups exactly as if they had clung together in being discharged from the lining of the alveolus. Nuclei and fragments of cells are also found which apparently belong to these epithelial cells.

2. Histologists recognize that it is a perfectly normal thing for both types of these cells to occur in the secretions of glands. For example, such cells frequently and almost constantly appear in saliva and in urine. White blood corpuscles normally make their way out through the walls of the capillaries into the connective tissues, and frequently make their way into the epithelial lining of the intestine, trachea, and other parts of the body. It is also normal to have epithelial cells discharged from epithelial surfaces, e.g., the lining of the mouth, and especially so in an active secreting surface such as is found in the alveoli of the udder. The secretion of the sebaceous glands is entirely composed of modified epithelial cells.

3. There is nothing in the appearance of histological preparations of the udder to justify the unusual interpretations which have been put upon the nature of these cells. When one studies preparations from udders which are known to have been actively discharging these cells at the time the animal was killed, there is no difficulty in finding white blood corpuscles abundantly in the connective tissues close about the alveoli. Similar cells, which stain in the same characteristic fashion, are found in the secretion

in the interior of the alveoli. Rarely, cells of the same type are found imbedded in the epithelium of the alveolus where they would be found if killed in the act of penetrating the lining of the alveolus. Free nuclei and entire cells of the same appearance as those found in the epithelial lining of the alveolus are also occasionally found in the interior of the alveoli, as they would be if detached from the secretory lining of the alveolus.

There is a continuous flow of large quantities of fluid from the lymph and blood vessels of the udder through the epithelial lining into the lumen of the alveolus in the process of milk secretion, and it is not at all surprising that the actively ameboid white blood corpuscles make their way through this epithelium with this current of fluid.

#### NORMAL NUMBER OF THESE CELLS FOUND IN MILK.

Before attempting to explain the significance of the presence of these cells it is necessary to know not only the nature and origin of the cells, but also the normal numbers and variations in numbers which occur.

The results obtained in counting the cells by the use of the method mentioned above have been much higher than those obtained by the examination of centrifuge slimes. At this time it is not possible to give the details of the examinations which have been made, but a brief outline may be given.

These examinations were first begun in Boston in 1910.<sup>1</sup> At this time 46 samples of milk from as many different herds in the neighborhood of Boston were found to contain an average of 1,485,000 cells per c.c. Eight market milk samples contained an average of 2,850,000 cells per c.c.

The following year<sup>2</sup> one of my students at Allegheny College, continuing the work, examined the milk of 37 cows from herds near Meadville, Pennsylvania, and found an average of 1,165,000 cells per c.c. He found great variation in the number of cells in the milk from different quarters of the udder. The largest number of cells occurred in the strippings. There was no constant relationship between the number of cells in the fore milk and that drawn during the latter part of the milking. This work was continued by the author at Göttingen, Germany, where the milk of 3 normal cows was examined daily for a period of 6 weeks. The daily variations in numbers of cells were found to be very large. One of these cows which was apparently normal in all respects was found to have very high cell counts; the highest one noted was

<sup>1</sup> *Jour. Infect. Dis.*, 1910, 7, p. 632.

<sup>2</sup> *Ibid.*, 1911, 8, p. 361.

5,975,000 per c.c. Individual quarters of the udder were tested on the same day and one quarter was found to be largely responsible for the high cell count. The milk from this quarter contained 22,500,000 cells per c.c., and yet this milk showed no evidence of being abnormal either in chemical analysis or in appearance. The cow was not suffering from garget, nor was a veterinarian able to find anything abnormal in her udder.

Numerous tests have been made of the herd at the experiment station at Geneva, New York. The average number of cells found in the milk of the 25 cows in the herd is 439,000 cells per c.c. Six cows have been studied in detail for periods of several weeks each. Two of these cows were fresh cows, 2 were abnormal because of abortion or troubles with the udder, and 2 were normal. A single examination has been made of the milk of each of 53 Guernsey cattle. The milk of these cows showed an average of 895,000 cells per c.c.

The data are presented in Table 1.

TABLE 1.

NO. OF COWS IN HERD	LOCATION	AVERAGE NUMBER OF CELLS PER C.C.	NUMBER OF COWS GIVING COUNTS			BREED OF COW
			Between 0 and 500,000	Between 500,000 and 1,000,000	1,000,000 and up	
37.....	Meadville	1,165,000	10	12	15	Jersey and mixed grades Harz and Glaner Jersey and grade Jersey Guernsey
3.....	Germany	932,000	1	1	1	
25.....	Exper. Station	439,000	18	6	1	
53.....	Geneva	895,000	27	16	10	
118.....			56	35	27	Totals
Average 884,000 cells per c.c.						

There is no evident explanation of the striking differences between these herds. They may be chance differences or they may have some significance. Evidently it is not safe to generalize without more complete data.

It would be interesting to analyze these data with a view to discovering whether the 27 cows in these herds whose milk showed counts above 1,000,000 per c.c. had any characteristics in common whereby they differed from the animals whose milk contained fewer cells. Unfortunately, however, the records at hand do not justify any such discussion. The work thus far done is only in the nature of a preliminary survey and the histories of these animals are not known as completely as is necessary for such a discussion.

#### CONCLUSIONS.

From studies thus far made, certain conclusions may be drawn, some of which may be regarded as established while others are only tentative and are drawn up at this time merely in order to summarize our information to date.

1. Normal milk contains cells derived from the body of the cow which are of two entirely different types: (a) white blood corpuscles which are largely of the polynuclear and polymorphonuclear type; these cells make their way into the milk by passage through the epithelial lining of the secreting portion of the gland, possibly, also, through the epithelial lining of the ducts of the gland; (b) epithelial cells, nuclei, and cell débris discharged from the epithelial lining of the secreting portion of the gland and possibly also from the ducts.

2. The number of these cells in apparently normal milk is exceedingly variable even in the milk from the same cow. The variation in the number of cells in the milk from the different quadrants of the udder is almost as great as the variation in number of cells in the milk of different animals. Apparently the strippings always contain a greater number of cells than the milk from the earlier part of the milking.

3. It is very common to find milk that contains so few cells that they can scarcely be counted with the method of examination used, i.e., less than 5,000 per c.c., but milk containing one or more millions of these cells per c.c. is met with frequently. The highest cell count which has been found in this work was in the case of the strippings from one quarter of the udder of a cow 8 days after calving where the milk showed the enormous count of 54,300,000 cells per c.c. Nevertheless, this milk was of an entirely normal appearance and careful bacteriological examination of the udder showed no evidence of streptococcic infection. This milk had an entirely normal taste and caused no evil after-effects.

4. Out of 122 individual cows whose milk has been examined, 59 have been found to give cell counts under 500,000 per c.c., 36 gave counts between 500,000 and 1,000,000 per c.c., and 27 gave cell counts over 1,000,000 per c.c. The milk of all of these cows was normal in appearance and was sold or used by their owners, who had every reason to suppose that the milk was normal milk.

5. There are no satisfactory data at hand which show whether there is, or is not, a relation between high cell counts and any of the following: streptococcic infection of the udder or other patho-

logical conditions of the udder, colostrum milk, milk from cows that are nearly dry, in heat, or in poor condition of flesh, etc. Such evidence as we have indicates that it is not at all likely that any of these conditions may be recognized by cell counts alone, especially in samples of market milk which consist of a mixture of the milk from several cows.

To answer the question implied in the title of this paper, "The Sanitary Significance of the Body Cells in Milk": it is entirely possible that some of the striking variations in numbers have a sanitary significance, as pathological conditions would certainly affect the discharge of these body cells. It seems probable, however, that the change produced by pathological conditions may not always be an increase in numbers of cells. Pathological disturbances in the blood are indicated just as surely by a decrease in numbers of red or white cells as by the reverse condition. Inasmuch as we have but very few data as yet on which to base conclusions, it is therefore impossible to make even a guess as to the final conclusions regarding the significance of the variations in number and character of the cells. The cells certainly do not have the significance of pus cells under ordinary conditions nor does it seem probable that it will be possible to recognize the admixture of pathological with normal milk by means of these cells alone. With our present knowledge it is certainly impossible to make this distinction.